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Proceeding Paper

Ozone Effects on the Quality of Swiss Chard. Peri-Urban Crops a Case Study †

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Abstract: The results of this open-top chamber study show that the variety of chard cv. Fuenlabrada, cultivated on the outskirts of Madrid, can be considered moderately sensitive to ozone. A significant loss of marketable production, the alteration of pigment concentration and alteration of nutritional quality was measured in the plants exposed to different ozone levels. Swiss chard has a high nutritional value due to its high macronutrient content and the presence of micronutrients essential for human health. The results of this study showed a significant reduction in the concentration of Mg and Ca in commercial chard leaves of the local variety Fuenlabrada. These results suggest that ozone can induce a loss of nutritional quality in leafy crops with negative consequences for human health.

Keywords: ozone effects; global change; Swiss chard quality; nutrients; leafy crops

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1. Introduction

Tropospheric ozone (O_3) is an important greenhouse gas. It is considered one of the most damaging air pollutants to vegetation due to its phytotoxicity and prevalence at high concentrations over large areas across the globe [1–5].

The photochemical pollution in Southern Europe is a subject of great environmental importance [6]. Despite the considerable improvements in air quality during the last decade in Europe, 80% of the urban areas were exposed to ozone levels exceeding the WHO guidelines. A recent study on ambient air quality in Spain showed a substantial improvement in the last 25 years, with the exception of tropospheric ozone [7]. The same trend was observed in the Madrid air basin [8]. During the ozone episodes in summer, high ozone concentrations in rural and urban stations were recorded [9,10].

The ozone impact assessments for crops were mainly based on modelling conducted for changes on crop yield. The estimated global impact of O_3 on crops suggests a yield reduction in major crops by 3–7%, causing high economic losses [11–14]. However, most estimates of the impact of current O_3 levels on crop production do not include horticultural production. These crops cover small areas, but they are of great economic importance.

Horticultural crops have been identified as extremely sensitive to O_3 damage [1,15–18]. Ozone causes a wide variety of damage to agricultural crops, including visible damage, reduced photosynthesis, alterations in carbon allocation, and a reduction in the quantity and quality of yield [1,17,19–21]. Visible leaf damage has been recorded in leafy crops, in some cases reaching a total loss of harvest in the field [15,22,23]. The effects of chronic exposure to O_3 on physiology or performance were studied for species such as lettuce, spinach or palak [24–30]. However, little information is available about the risk of ozone effects on quality traits.

Swiss chard is consumed all over the world, due to its organoleptic properties as well as its numerous benefits for human nutrition [31]. In addition, chard has a high nutritional importance as it contains fiber, vitamins A C E K B and minerals (calcium, iron, zinc phosphorus, magnesium, potassium, iron and manganese) [32–34].

Cumulative metrics for O_3 exposure have been used along with estimations of the minimum thresholds for damage in order to estimate crop yield losses due to O_3. Ozone threshold values (critical levels) were first established within the framework of the Convention on Long-Range Transboundary Air Pollution (CLRTAP, UNECE) for the protection of different vegetation types [35]. These critical levels are the basis for defining the objective values for plant protection in the European Air Quality Directive (2008/50/EC) and are the main tools for O_3 risk assessment studies.

2. Material and Methods

2.1. Experimental Field Facilities and Plant Material

The ozone fumigation experiments were carried out in an open-top chamber (OTC) facility located in central Spain: Finca Experimental La Higueruela (CSIC) (Santa Olalla, Toledo, 450 m.a.s.l.; 40°3′ N, 4°26′ W). Swiss chard plants were exposed to four O_3 treatments: charcoal filtered air (FA) with over 50% filtration efficiency, which kept O_3 levels below background concentrations; non-filtered air (NFA), reproducing current ambient levels; non-filtered air supplemented with 20 nL L^{-1} O_3 (NFA+); and non-filtered air supplemented with 40 nL L^{-1} O_3 (NFA++). Additional O_3 supply for NFA+ and NFA++ treatments was applied during 8 h per day, 7 days a week using an O_3 generator system (Model 16, A2Z Ozone Systems Inc., Louisville, KY, USA) fed with pure oxygen. The experiment followed a random block design with four O_3 treatments, each replicated three times (three OTCs per O_3 treatment). More details about the facility are provided in Calvete-Sogo et al. (2014).

The study was carried out on a native variety of chard (*Beta vulgaris* L. var *cicla*) from Fuenlabrada. The seeds were grown according to greenhouse standards until the plants were large enough for transplantation. Seedlings were transplanted to 2.5 L pots with a mix of unfertilized blond peat (60%), perlite (20%) and vermiculite (20%). Fertilization was carried out with Nutricote (NPK:18-6-8) at the time of transplanting. In each chamber, 4 plants were exposed to different ozone treatments for 43 days (from 4 April to 25 May).

A risk assessment study of ambient ozone effects on a native variety of chard, cv. Fuenlabrada, was carried out at a peri-urban agricultural area: Parque Agrario de Fuenlabrada (Fuenlabrada, Madrid, 660 m.a.s.l., 40°17′ N, 3°51′ W). The chard trial was carried out with organic mulch on the surface before planting (4 kg/m^2), and then triple 15 fertilizer (150 kg/ha) was added. Equlibrium and Nutriseck were applied as a pest preventive method. Irrigation was carried out by a sprinkler, the most suitable method for the sandy soil of the area. Plant growth from transplantation to harvest in late summer was 43 days.

In parallel, the impact of ambient ozone levels was investigated through the quantification of visible lesions in the leaves of O_3-sensitive and -tolerant varieties in an O_3 bioindicator garden. The experimental design of the ozone garden was carried out using ozone tolerant/sensitive variety pairs. Six species were used (tomato, chard, bean, watermelon, tobacco and wheat) with a total of 24 varieties. Environmental growing conditions and ozone levels were collected from the Air Quality Network of the Community of Madrid (Fuenlabrada Station).

2.2. Physiological Measurements

2.2.1. Biomass

The biomass production was determined at the maximum development of the plant. All the leaves were collected and classified as commercial and non-commercial biomass. This classification was based on the absence or presence of lesions on the leaves. The fresh weight (FW) was determined for the total plant. A known FW sample from each category was dried in a ventilated oven at 65 °C for 48 h to obtain the dry weight (DW). Data were analyzed based on total biomass and commercial biomass per plant.

2.2.2. Foliar Pigment Content

The content of chlorophyll, polyphenols (anthocyanins, flavonols) and the nitrogen balance index (NBI®) were measured with DUALEX® (Force-A, Orsay, France) in fully developed leaves in the external position of the plant. Four plants were measured by OTC.

2.2.3. Nutrient Analysis

For nutrient analysis, oven-dried samples from the final harvest were ground in a stainless steel grinder. Elemental composition analysis was performed by an elemental analyzer (TruSpect CHN-S, LECO Corporation, St. Joseph, MI, USA). Nutrients composition (Mg, Ca, Na, K, Fe, Mn and Zn) was analyzed by an ICP-OES analyzer (5900 ICP-OES, Agilent, Santa Clara, CA, USA).

The analyses were carried out using 150 mg of commercial biomass per plant. Four plants per OTC. The values were expressed as mg/100 g DW.

2.2.4. Statistical Analysis

All statistical analyses were conducted using Statistica (TIBCO, Palo Alto, CA, USA). Differences among O_3 treatments in physiological parameters were tested through one way analysis of variance (ANOVA). A probability level $p < 0.05$ was considered statistically significant and p values between 0.05 and 0.1 were discussed as significant trends.

3. Results

3.1. Growing Conditions and Ozone Concentration

The growing conditions in OTC were sunny and warm. The temperature range was 8–21 °C, and the relative humidity was 39–80% (Table 1), while the environmental conditions in the peri-urban crops were warmer and drier in the summer months. The ambient temperature was in the range of 16–33 °C, and the relative humidity was 12–57% (Table 1).

Table 1. Environmental growing conditions and ozone exposure in OTC and a peri-urban field. Charcoal filter air (FA), ambient (NFA), ambient plus 20 ppb ozone (NFA+) and ambient plus 40 ppb ozone (NFA++).

Year	Ozone Exposure (Days)	(O_3 ppb) [a]-AOT 40 (ppb·h)					
OTC		**FA**	**NFA**	**NFA+**	**NFA++**	**RH (%) [a]**	**T (°C) [a]**
2019	25	(17)-0	(36)-1869	(42)-4084	(49.5)-7158		
	43	(17)-0	(36)-3550	(42)-8075	(49.5)-13809	59	16
Peri-Urban Field		**Environmental O_3 Concentration**					
	Summer (JJA)	(38)-10459				29	25
	43	(40)-5840				25	25.5

T: air temperature; RH: air relative humidity; (O_3 ppb): ozone concentration; AOT40: accumulated ozone concentration over 40 ppb during daylight hours. [a] Averages 24 h.

The concentration of ambient ozone recorded in peri-urban crops exceeded the critical level of vegetation protection (3000 ppb·h). Ozone peaks throughout the day were recorded between 2:00 p.m. and 6:00 p.m., coinciding with the highest values of temperature and lowest relative humidity throughout the day.

3.2. Visible Injury

The plants of Swiss chard var. Fuenlabrada showed an unspecific discoloration after 25 days of treatment in the OTC. These white spots were more intense in the NF++ treatment. At the end of the experiment, these spots in ozone treatments were less apparent.

In the O_3 bioindicator vegetable garden, no visible damage was detected in the chard varieties. Ozone damage was only identified in a variety of sensitive tobacco (Bel W3) (data not shown).

3.3. Biomass

The total production of chard was similar between different treatments. When the marketable production was selected, a significant reduction in the harvest was detected in the ozone treatments ($p < 0.01$) and a significant increase in non-commercial biomass in NF++ treatment ($p < 0.05$). The harvest of chard cv. Fuenlabrada was reduced in 30% in the NF++ and 17% in the NF+ treatment compared to the filtered air treatment harvest (Figure 1).

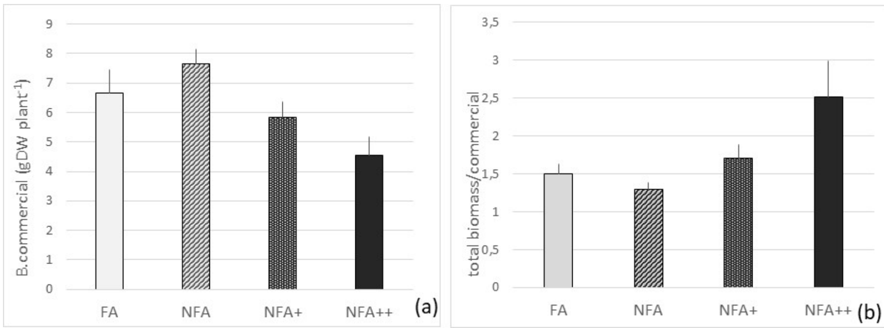

Figure 1. Swiss chard (**a**) commercial biomass (g dry weight plant^{-1}); (**b**) total biomass:commercial ratio. Values are means ± standard errors for ozone treatments. Charcoal filter air (FA), ambient (NFA), ambient plus 20 ppb ozone (NFA+) and ambient plus 40 ppb ozone (NFA++).

3.4. Pigments Concentrations

Pigment analysis at 25 days of the experiment showed a 15% reduction in chlorophylls in the NF++ treatment ($p < 0.01$) (data not shown). At the end of the experiment (after 43 days of exposure to different concentrations of ozone), the leaves of plants in NF++ treatment showed a significant reduction of 15% ($p < 0.001$) in chlorophyll concentration. They also showed a reduction of 15% in the nitrogen index (NIB) ($p < 0.01$) and an increase of 10% in the concentration of anthocyanins ($p < 0.001$) in plants exposed to a higher concentration of ozone (NF++), while no differences were obtained in flavonoids (Figure 2). Measurements with "Dualex" in peri-urban crops at different foliar ages showed a significant reduction in flavonoids ($p < 0.001$) and an increase in NBI in the youngest leaves ($p < 0.001$), located in the center of the plant.

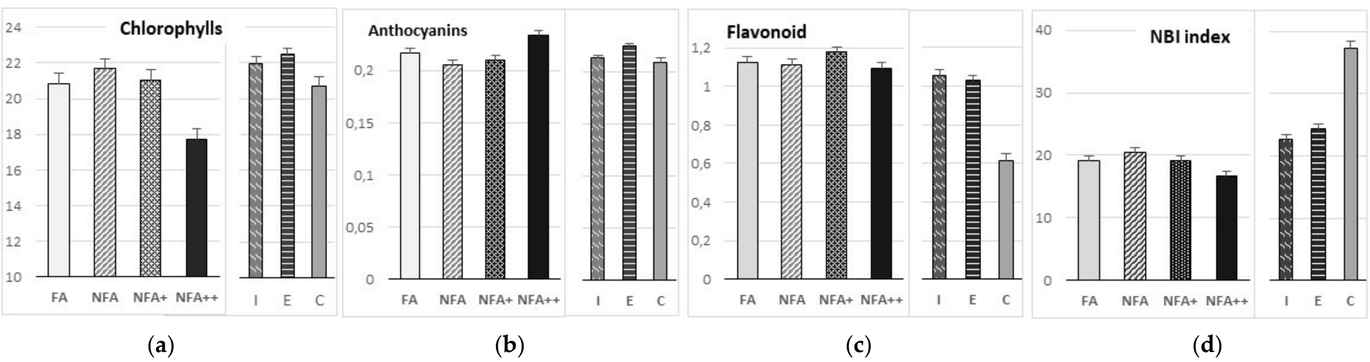

Figure 2. (**a**) The content of chlorophyll, (**b**) anthocyanins, (**c**) flavonoids and (**d**) the nitrogen balance index (NBI) was measured with a Dualex device inside the OTC and in the peri-urban field. Values are means ± standard errors for ozone treatments. The ozone treatments are: air with carbon filter (FA), ambient (NFA), ambient plus ozone 20 ppb (NFA+) and ambient plus ozone 40 ppb (NFA++). Those measured in the field were classified according to the age and position of the leaf. E = external, I = internal and C = center.

3.5. Nutrient

The main nutrients in the chard leaves grown in the OTC were K, Na, Mg and Ca (Table 2). At the end of the experiment, a significant reduction in Mg of 25% and Ca (30%) ($p < 0.05$) was observed in the NF++ treatment plants. Additionally, a non-significant reduction in Mn of 10% and in Zn of 5% was observed in the same treatment. The concentration of K was significantly higher in the plants of the NF++ treatment ($p < 0.001$) with respect to NF and NF+ but not with respect to FA. The concentration of other nutrients, such as nitrogen (N) or phosphorus (P), increased slightly with the increased exposure to ozone (Table 2).

Table 2. *p*-values of ozone effects on nutrient levels and nutrient concentrations (mg/100 g DW) in commercials leaves after 43 days of exposition to different ozone treatments. Values are means ± standard errors for ozone treatments. The letters denote differences between ozone treatments in the post hoc tests n.s. = not significant. The ozone treatments are: air with carbon filter (FA), ambient (NFA), ambient plus ozone 20 ppb (NFA+) and ambient plus ozone 40 ppb (NFA+).

Factor	N	Mg	Ca	K	Na	P	Fe	Mn	Zn	Cu
O_3	n.s.	0.02	0.03	0.01	n.s.	n.s.	n.s.	n.s	n.s.	n.s.
FA	3482 ± 160	1370 ± 57 a	877 ± 66 a	3690 ± 137 ab	3500 ± 135	256 ± 17	21 ± 2.4	17 ± 0.9	9.16 ± 0.39	2.59 ± 0.20
NFA	3336 ± 171	1229 ± 105 b	770 ± 91 a	3145 ± 163 b	3636 ± 164	240 ± 7.6	18 ± 1.8	18 ± 1.1	9.77 ± 0.66	2.44 ± 0.22
NFA+	3354 ± 190	1236 ± 127 b	727 ± 106 ab	3160 ± 231 b	3727 ± 201	253 ± 21	19 ± 1.3	16 ± 1.5	8.63 ± 0.91	2.37 ± 0.19
NFA++	3836 ± 200	960 ± 60 b	541 ± 38 b	3836 ± 143 a	3240 ± 93	287 ± 15	17 ± 0.9	15 ± 1.2	8.45 ± 0.53	2.75 ± 0.17

The variation of Mg with respect to the exposure to ozone showed a high correlation with the changes in the concentration of chlorophylls ($R^2 = 0.9$) measured after 43 days of exposure.

4. Discussion

The results of this study in the OTC show that the variety of chard cv. Fuenlabrada cultivated on the outskirts of Madrid can be considered moderately sensitive to ozone. The alteration in the concentration of pigments was detected earlier, with a lower accumulated ozone exposure. As a result, a significant loss of marketable production and an alteration in nutritional quality was measured at the end of the experiment in the plants exposed to high ozone levels.

Usually, leafy crops with visible damage are often not marketable; consequently, the production of leaf crops is reduced where ambient O_3 concentration reaches high levels [15,18,36,37]. Yield quality traits are studied in a limited number of crops.

The alterations in pigment concentrations were recorded after ozone exposure in numerous studies [20], and the reduction in chlorophyll was detected in leafy crops [27,28]. These changes were more evident in old leaves and were dependent on the variety studied [37]. Other studies showed a seasonal response [29]. The earlier chlorophyll breakdown could be related to a premature and accelerated senescence due to ozone [21,38]. Enhanced senescence will also reduce photosynthesis and the length of time over which nutrients will be extracted from the soil [39]. The response of increased anthocyanin concentration measured in the plants exposed to high ozone leaves has been linked to the response to oxidative stress.

Chlorophyll reduction is not a specific effect of ozone. A low concentration of chlorophyll is also observed in young leaves. The values obtained from anthocyanins, flavonoids, or NBI index measured with a "Dualex device" could help to discern between the effect of ozone and the age of the leaf. Low values of flavonoid and high-index NBI values (measured with Dualex) were related to less leaf development. This same pattern was observed in tobacco plants grown in the bioindicator garden.

The chlorophyll content in chard leaves was highly correlated ($R^2 = 0.9$) with the concentration of Mg in these plants. Both traits showed a significant reduction in NF++ treatment. These measurements were determined on green leaves without visible symptoms. A lower correlation value was recorded with Ca and Fe. However, an inverse relationship with nitrogen concentration was detected. Plants with a lower concentration of chlorophylls have a higher concentration of nitrogen. However, the differences in N concentration were not significant. The increase in nitrogen with exposure to ozone has been reported in different studies, especially in wheat grains. This means that grain quality is improved with respect to its concentration, but the amount of accumulated proteins and minerals per unit area is reduced, which can have serious effects on human nutrition [39].

Chard has traditionally been used for its health benefits [31,32]. Regarding the mineral content, the abundance of macro elements, such as Mg, Ca or K, and the presence of the microelements Fe, Cu, Mn and Zn, are some of the most interesting aspects of the use of this crop in human nutrition. Mineral deficiency is one of the causes of numerous chronic and degenerative disorders. The consumption of a plate (200 g) of fresh Swiss chards leaves could cover around 20% and 40% of Fe, 100% Mg and 15% of Ca (RDA) (recommended dietary allowance). Exposure to ozone reduces the concentration of Mg, Ca or Fe by 5–20%, causing a reduction in RDA in the diet. A similar reduction in nutrients was recorded in palak when the plants were exposed to ozone in winter, but no differences were recorded in summer [29]. The reductions in Mg, Ca, Fe and Zn were related to the increasing senescence in the exposure of the leaves to ozone [38].

The OTC results in chard cv Fuenlabrada showed that this cultivar was moderately sensitive to ozone. The first effect of ozone (chlorophyll reduction) was detected with the accumulated ozone concentration in the range of 4000 ppb·h (AOT40). A reduction in marketable biomass and a moderate loss of quality was detected with a cumulative ozone concentration of 13,800 ppb·h (AOT40).

Ozone effects were not clearly detected in the field. No visible symptoms or reductions in growth were detected, and pigment concentration values were in the range of healthy plants. Different results could be related to the meteorological conditions and to lower ozone exposures during the growth of the crop. In the OTC facilities, the conditions were milder than in the peri-urban field, where very low relative humidity values were recorded in the summer months, coinciding with the maximum ozone concentration. Drier conditions could reduce the absorption of pollutants [20,21].

The evaluation of O_3 pressure on global agriculture should include quality and nutritional aspects with a direct consideration of the potential for adverse health effects and malnutrition associated with the reduced production of proteins and a variety of minerals.

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